Biological Metal Clusters: Biophysical and Model Studies (2)

M. Maroney, K. Bose, G. Davidson, F. Al-Mjeni, P. Carrington (U. of Massachusetts) J. Honek and S. Clugston (U. of Waterloo)

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Glyoxalase I (Glx I) is part of a two enzyme system that is involved in detoxification of methylglyoxal. Methylglyoxal is a cytotoxic compound and has been demonstrated to be both a mutagen and a carcinogen. Glx I is an isomerase that serves to detoxify methylglyoxal by catalyzing the conversion of the hemimercaptal formed non-enzymatically from methylglyoxal and glutathione to the thioester of *D*-lactate. All Glx I enzymes studied to date require a metal for activity. The human, yeast and *Psudomonas putida* bacterial enzymes are active in the presence of Zn, while the *E. coli* GlxI is activated by Ni and is inactive with Zn bound. We have collected XAS data on native Glx I, Zn-substituted Glx I, and complexes formed with S-*D*-lactoylglutathione (enzyme-product complex) S-Octylglutathione and hydroxamate (inhibitor complexes). We also have data on a protein engineered with SeMet near the active site. The first paper, which characterizes the native Ni site structure and compares it with the Zn substituted enzyme, is in press (Davidson, G., Clugston, S. L., Honek, J. F., Maroney, M. J. *Inorg. Chem.* 39: 2000). This study shows that the Ni site is six-coordinate and bound to two carboxylate, two histidine imidazoles and two other O/N donors (presumably water molecules). Identification of the histidine imidazole and carboxylate ligands employed multiple scattering analysis. The data confirms the ligand environment suggested by sequence comparisons between human Glx I and the *E. coli* enzyme. In particular, it confirms that the His residue that substitutes for a Gln ligand in the human enzyme is a Ni ligand. However, this substitution is not responsible for the metal specific activity, since many bacterial enzymes that are active with Zn have the same substitution. Comparison of the structure of the Zn-substituted enzyme and the native enzyme reveals that the Zn is five-coordinate with a ligand environment composed of 2 His, 2 carboxylate and one O/N (*e.g.* water) ligands, suggesting that one possible reason for the inactivity of the Zn-substituted enzyme is that both water molecules are required. Analysis of the data from the various complexes is in progress and will be the focus of a second manuscript.